

No	Structure	$pl_{50}(\text{Protox})^a$	S_{BC}^b	S_{CD}^c
27		8.89	0.80	0.63
28		8.66	0.83	0.64
29		6.70	0.85	0.65
30		7.82	0.85	0.67
31		7.72	0.86	0.68

Table 2. Protox inhibition and shape similarity index of diphenyl ethers

^a $pl_{50}(\text{Protox}) = -\log [\text{molar } I_{50} \text{ of protox isolated from corn}]$.

^b Shape similarity index between diphenyl ethers and B and C ring of protox.

^c Shape similarity index between diphenyl ethers and C and D ring of protox.

Atoms	Degree	Atoms	Degree
A-B rings		C-D rings	
C1-C6-C11-C12	−123.60	N25-C24-C21-C20	47.30
N7-C6-C11-C12	53.40	C26-C24-C21-C20	−132.90
C6-C11-C12-N13	60.40	C24-C21-C20-N17	43.00
C6-C11-C12-C15	−116.70	C24-C21-C20-C19	−139.20
B-C rings		D-A rings	
N13-C14-C22-C23	−72.40	N17-C10-C9-C8	−47.40
C16-C14-C22-C23	106.30	C18-C10-C9-C8	133.00
C14-C22-C23-N25	−34.50	C10-C9-C8-N7	−53.80
C14-C22-C23-C27	147.40	C10-C9-C8-C4	129.10

Table 3. Torsion angles in the most stable conformation of the protoporphyinogen IX

cyclic imides. This superimposability is one of the very important factors in mimicking the substrate. There was a significant correlation between the protox-inhibiting activity and the similarity index of cyclic imides as shown by eqn (2), in which n , r , s and F represent the number of data, the correlation coefficient, the standard deviation and the ratio of regression and residual variances, respectively, and the figures in parentheses are within the 95% confidence interval.

$$pI_{50}(\text{Protox}) = 20.3(\pm 3.31)S_{CD} - 8.10$$

$$(n = 26, r = 0.93, s = 0.46, F_{1,24} = 160.5) \quad (2)$$

These results indicate that structural similarity is a very important factor in the recognition of protox between inhibitors and the substrate substructure. These comparative experiments show that cyclic imides and diphenyl ethers inhibit the protox, but the binding sites for both herbicide classes in the protox enzyme are different.³

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Structure and fungicidal activities of methoxyiminophenylacetamide derivatives

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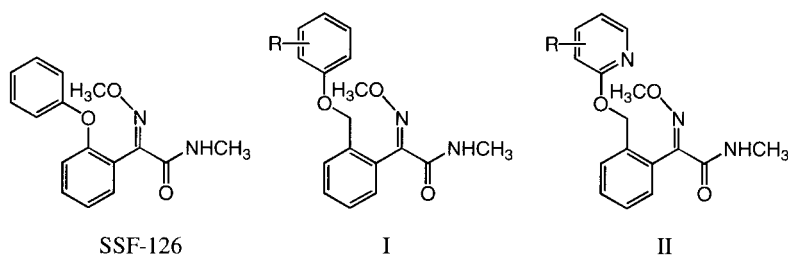
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Abstract: Methoxyiminoacetamides derived from ring-cleaved isoxazole compounds are active against economically important fungal diseases of many crops. Our approach, based upon biorational design, resulted in the production of methoxyiminophenylacetamide derivatives similar in their chemical

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Figure 1. Structure of SSF-126 and 2-phenoxyethylphenyl or 2-pyridyloxymethylphenyl derivatives of methoxyiminoacetamide.



structure and biological performance to a family of strobilurins produced by *Basidiomycotina* fungi.

A number of 2-methoxyimino-2-(2-phenoxyphenyl)acetamide derivatives were synthesized and their fungicidal activity evaluated from which, (*E*)-2-methoxyimino-*N*-methyl-2-(2-phenoxyphenyl)acetamide (SSF-126) was selected as a promising candidate blasticide.

Further studies revealed that the substitution of a substituted phenoxyethyl group for the phenoxy group contributed to an increase in the fungicidal activity. Mono-, di- or tri-substitutions at 2-, 3-, 4- and/or 5-positions of the benzene ring by halogen or lower alkyl also led to an increase in the activity. However, 2,6-di- or 2,3,6-tri-substituted derivatives were less active against the crop diseases tested. Furthermore, the phenoxyethyl group could be replaced by the 2-pyridyloxymethyl group. Mono- or di-substitutions at 3-, 5-, and/or 6-positions of the pyridine ring by Cl or CF₃ resulted in a good control of the crop diseases tested.

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Keywords: Methoxyiminophenylacetamide derivatives; fungicidal activity; strobilurin analogues; isoxazole; SSF-126; rice blast; gray mold; cucumber powdery mildew

An antibacterial agent, Sinomin[®], and a herbicide, isouron, have been developed as a result of research on isoxazole derivatives in our company. During the course of metabolic studies of the latter, a ketoena-

mine derived from the cleavage of the isoxazole ring was obtained as a metabolite under paddy field conditions.¹ We designed ring-cleaved derivatives of the isoxazolecaboxamides, which showed fungicidal activity against rice blast. Some α -imino or α -ketoamide derivatives showed no fungicidal activity, but the alkoxyimino ketoamide derivatives showed good activities against rice blast, cucumber gray mold and cucumber powdery mildew.²

A number of 2-methoxyimino-2-(2-phenoxyphenyl)acetamides were synthesized and their fungicidal activities were determined. Among them, SSF-126[†] ((*E*)-2-methoxyimino-*N*-methyl-2-(2-phenoxyphenyl)acetamide; Fig. 1) was selected² and developed as a blasticide. Further studies revealed that the substitution of a substituted-phenoxyethyl group for the phenoxy group (1; Fig 1) contributed to an increase in the fungicidal activity. Preventive activities of methoxyiminophenylacetamide derivatives on rice blast, wheat powdery mildew, cucumber powdery mildew, cucumber gray mold and cucumber downy mildew were assessed in foliar application tests. Test compounds were dissolved in a small amount of *N,N*-dimethylformamide and diluted to a given concentration with distilled water containing a sticking agent. The solutions or suspensions of the test compound were sprayed onto the

[†] Proposed BSI/ISO common name metaminostrobin.

Compound	<i>R</i> ^b	Rice blast	Wheat powdery mildew	Cucumber		
				Powdery mildew	Gray mold	Downy mildew
SSF-126		4	3	3	3	3
I	H	3	3	2	3	3
I	2-Me	4	4	4	3	4
I	2,5-Me ₂	4	4	4	3	4
I	2,6-Me ₂	1	1	1	1	0
I	2,3,5-Me ₃	3	4	4	4	4
I	2,3,6-Me ₃	1	0	0	0	0
II	H	0	3	3	2	2
II	3-CF ₃	4	4	3	3	2
II	3-CF ₃ 5-Cl	3	4	4	3	4
II	3-CF ₃ 6-Cl	3	4	4	3	4
II	3-Cl-5-CF ₃	3	4	4	4	4

Table 1. Fungicidal activities^a of methoxyiminophenylacetamide derivatives

^a Fungicidal activities were expressed on a scale 4, 3, 2, 1, corresponding to a c90% control at doses of 2.0, 7.8, 31.3 or 125 mg litre⁻¹, respectively, or 0, representing little or no activity.

^b See figure 1.

host plant at 24 h before inoculation. Inoculation was carried out by spraying the conidia of *Pyricularia oryzae* Cavara [rice blast, anamorph of *Magnaporthe grisea* (Hebert) Barr], *Sphaerotheca fuliginea* (Schlecht) Poll (cucumber powdery mildew) or *Erysiphe graminis* DC f sp *tritici* Marchal (wheat powdery mildew) onto the host plants, or by putting the mycelial mats of *Botrytis cinerea* Pers (cucumber gray mold), or dropping a zoosporangial suspension of *Pseudoperonospora cubensis* (Berk & MA Curtis) Rost (cucumber downy mildew) onto the leaf surface of cucumber seedlings. The results in Table 1 suggested that the substituents on the benzene ring play an important role in fungicidal activity. Mono-, di- or tri-substitutions at the 2-, 3-, 4- and/or 5-positions of the benzene ring by halogen or lower alkyl also led to an increase in the activity. However, 2,6-di- or 2,3,6-tri-substituted derivatives were less active against all diseases tested.

Furthermore, the phenoxymethyl group could be replaced with a 2-pyridyloxymethyl group (11, Fig 1). Mono- or di-substitutions at the 3-, 5- and/or 6-position of the pyridine ring by Cl or CF₃ resulted in excellent control against a wide range of diseases of upland crops by foliar application.

Strobilurin analogues are a new class of fungicide with broad-spectrum activity and a new mode of action.^{3–5} They inhibit the bc₁ segment of the respiratory chain of mitochondria.⁶ SSF-126 also inhibits the same target site of mitochondria.⁷ During the optimization process with the methoxyiminophenylacetamide derivatives, we noted the similarity of the isoxazole ring-cleaved compounds to the strobilurin analogues, derived from strobilurin A which is produced by a species of Basidiomycotina. Consequently, the methoxyiminophenylacetamide derivatives obtained by the approach of the ring-cleavage design of isoxazole are strobilurin analogues from the viewpoints of chemical structure and biological performance.

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Glutathione S-transferases in black-grass (*Alopecurus myosuroides* Huds.): Properties and involvement in herbicide resistance

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Abstract: Black-grass (*Alopecurus myosuroides* Huds) is a major problem weed in winter cereal crops in the UK. In recent years herbicide resistance has been detected in this species. Research suggests that this is due, at least in part, to increased herbicide metabolism. One group of enzymes implicated in herbicide metabolism are glutathione S-transferases (GSTs). In this study, GSTs have been purified from black-grass in order to investigate further their role in resistance. In addition, the effects of herbicides on GST activity *in vitro* have been studied.

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Keywords: herbicide resistance; black-grass; *Alopecurus myosuroides*; glutathione S-transferase; protein purification

Glutathione S-transferases (GSTs, E.C.2.5.1.18) are a group of enzymes which catalyse the conjugation of a wide range of substrates with the tripeptide glutathione (γ -Glu-Cys-Gly; GSH). GSTs occur throughout the animal and plant kingdom. In animals they have been extensively studied and have been implicated in many roles including toxin and drug metabolism. In plants, GSTs have been less well studied, but have been implicated in herbicide resistance, crop herbicide tolerance, various stress responses and in secondary metabolism, as recently reviewed by Marrs.¹ GSTs have been linked to the metabolism of a variety of herbicides and, in the case of atrazine, glutathione conjugates have been isolated.² With this in mind, we have studied the possible roles played by GSTs in herbicide resistance in

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